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ABSTRACTS
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(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Studies on the Chemical Constituents of "Inekoji." Part VII.

The Red Pigment, Ustilaginoidin (IV).

(pp. 1159~1161)

By Teijiro YABUTA, Yusuke SUMIKI and Kimiko ANNO.

(Tokyo Imperial University; Received November 20, 1940.)

On the Catalase in Juice of Fruits, Roots or Stems.

(pp. 1162~1166)

By Hisao MATUI.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received September 20, 1940.)

The catalase action of juice of fruits (about 30 sorts) and of tubers, bulbs, roots or stems of various vegetables was examined. The results may be summarized as follows:

1. The concentration of hydrogen ion in the medium in which the reaction is occurring influences the catalase action of fruit juice. The optimum concentration for the catalase action lies between pH 7 and 8 with some exceptions—5.8 (tomato), 6.0 (apple) and 8.6 (persimmon).

2. The catalase action of fruit juice is different in strength according to families of plants. Generally orange and grape juices are feeble and that of melons strong.

3. In general, hydrogen ion concentration of fruit juice has a great influence upon the catalase content. If pH value of juice is small, the catalase action is weak and according as pH value approaches 7 the catalase action becomes gradually strong, but a few exceptions are recognized.

4. Juice of bubers, bulbs, roots or stems of vegetables contains a larger quantity of catalase than juice of ordinary fruits.

On the Oxidative Substance Appearing in Juice of Salted Vegetables.

(pp. 1167～1168)

By Hisao MATUI.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received September 20, 1940.)

The existence of nitrite has been demonstrated in juice of salted vegetables (garden radish, turnip, cabbage and various greens). Whe the vegetable is salted, nitrite is detected in the juice by Griess' test in a few days, and this may be perhaps produced by bacteria (lactic acid bacterium, etc.) from nitrate which is contained in vegetable juice. The amount of nitrite often reaches 230.5 mg as N_2O_3 in 100 cc of salted juice. But it gradually disappears, as nitrous anhydride is formed in acidic condition.

On the Cellulose Analysis and Bleaching Methods of Cellulose Materials. Part IV.

Application of the Modified New Method of Cellulose Estimation on Various Plant Analyses.

(pp. 1169～1175)

By Sin-iti HONDA.

(Kyoto Imperial University;
Received October 19, 1940.)

In the previous papers, the present author proposed the new method, modification of Jenkins' original method. With the view to proving the general usefullness of the modified method the present author applied as an example this method for the cellulose analysis of several plant materials. The results are tabulated in Table I.

Table I. Comparison of plant cellulose contents by different analytical methods with various materials. (Oven dry state.)

Phase of chlorination. / Analytical method. / Compositions.	Liquid Phase (Bleaching powder solution)		Gaseous Phase
	Previous Method.	Modified Method	Cross and Bevan's Modified Method.
Hitujigusa (Poa glumaris, Tri.)			
Total cellulose (%)	47.52±0.46	44.77±0.03	44.10±0.46
α-cellulose (ash-free) (%)	34 45±0 89	33.10±0.09	32.55±0.88
In total cellulose: α-cellulose (ash-free) (%)	72.51±1.80	73.92±0.26	73 77±1.16
In total cellulose: α-cellulose (%) ash (%)	0.99	1.05	—
In total cellulose: β-cellulose (%)	7.75	25.03	25.29
In total cellulose: γ-cellulose (%)	19.75		
Number of chlorinations.	2N, 2A	3A	?

Goyō no matu (Pinus parviflora.)

Total cellulose (%)		56.03±0.06	55.29±0.43	52.55±0.09
α-cellulose (ash-free) (%)		39.12±0.08	39.02±0.28	34.37±0.41
In total cellulose	α-cellulose (ash-free) (%)	69.82±0.09	70.42±0.12	65.41±1.0
	α-cellulose ash (%)	0.23	0.33	—
	β-cellulose (%), γ-cellulose	30.02	29.25	34.59
Number of chlorinations.		2N, 4A	4A	?

Chosen Gōyo no matu (Pinus Koraiensis, Sieb et Zucc.)

Total cellulose (%)		52.44±0.12	51.56±0.36	49.92±0.06
α-cellulose (ash-free) (%)		37.97±0.47	37.10±0.52	30.03
In total cellulose	α-cellulose (ash-free) (%)	72.40±0.35	71.96±0.76	60.95
	α-cellulose ash (%)	0.04	0.34	—
	β-cellulose (%)	2.22	27.70 (β + γ)	17.87
	γ-cellulose (%)	25.34		21.18
Number of chlorinations.		2N, 5A	5A	?

Hosoba isotutuji (Ledum palustre L. var, vulgare Ledeb.)

Total cellulose (%)		35.32±0.48	35.47±0.10	
α-cellulose (ash-free) (%)		22.73±0.58	21.89±0.40	
In total cellulose	α-cellulose (ash-free) (%)	64.39±2.26	61.70±1.25	
	α-cellulose ash (%)	5.26	1.90	
	β-cellulose (%), γ-cellulose (%)	30.35	37.40	
Number of chlorinations.		2N, 6A	6A	

Sirakanba (Betula japonica Sieb. or B. latifolia Kom.)

(1) Sapwood.

Total cellulose (%)		57.93±0.88	58.06±0.37	
α-cellulose (ash-free) (%)		42.13±0.65	41.48±0.18	
In total cellulose	α-cellulose (ash-free) (%)	72.65+0.21	71.44±0.17	
	α-cellulose ash (%)	0.27	0.26	
	β-cellulose (%)	15.01	18.72	
	γ-cellulose (%)	12.07	9.78	
Number of chlorinations.		2N, 4A.	4A	

(2) Heartwood.

Total cellulose (%)		53.68±1.18	57.06±0.25	
α-cellulose (ash-frec) (%)		37.47±0.62	38.56±0.16	
In total cellulose	α-cellulose (ash-free) (%)	69.83±0.37	67.59±0.19	
	α-cellulose ash (%)	0.30	0.32	
	β-cellulose (%), γ-cellulose (%)	29.87	32.09	
Number of chlorinations.		2N, 3A	3A	

It will be seen that the total cellulose contents were always higher in the modified method than given in the original paper. However, the mean difference is about 1.5 %, and thus the modified method may be quite sufficient for use in the pulp and paper industries.

Moreover, with regard to the α-cellulose contents shown in Table I, the results of analysis with the modified method show good agreement with Jenkins' original method.

The experimental results by Cross & Bevan's chrolination method obtained in the present author's laboratory were also tabulated in Table I, for comparison.

Thus it was seen that Jenkins' original method may be used instead of Cross & Bevan's chlorination method. Moreover, the modified method proposed by the present author may be recommended as an improved and simplified method in place of Jenkins' original method.

(Prof. Sikata's Laboratory, The Institute of Chemical Research, Kioto Teikoku-Daigaku.)

On the Denaturation of Sericin. Part 3.

Some References to the Denaturation of $\alpha_{3.8}$-Sericin Powder with $\alpha_{4.4}$-Sericin Powder.

(pp. 1176~1180)

By Zirō Hirose.

1. Introduction.

In the previous paper[1], we studied isoelectric point of α-sericin and found isoelectric point of α-sericin in soluble sericin fraction is more alkaline than that of insoluble one, corresponding to their solubility.

In this paper we studied some references of denaturation of $\alpha_{3.8}$-sericin (obtained by Ito and Komori's method[2]) powder with $\alpha_{4.4}$-sericin (obtained soluble sericin fraction[1], powder stoichiochemically. But in this and further reports, designation of α-sericin, in details, was followed by the next example.

A. α-sericin, being precipitated at pH 4.4·········$\alpha_{4.4}$-sericin and so on.

B. α-sericin, being obtained as insoluble part when original α-sericin was boiled with distilled water for definite time,·········α_1-sericin. If we wish to show their isoelectric point,·········$\alpha_{4.2}$-sericin, and so on.

2. Experimental.

(1) Preparation and isolation of α-sericins.

(A) $\alpha_{3.8}$-sericin[2].

200 gs. of raw cocoons, being freed from chrysalid, extracted by boiling (110°C) with 6 *l*. of distilled water for 30 minutes. Extraction was repeated twice. All the extracts were collected, and to this sericin sol added acetate mixture of

pH 3.8 (final conc.⋯0.02 M). Precipitate thus formed was brought to the powdered state by means of alcohol and ether.

Yield,⋯⋯27.2 gs. N %,⋯⋯16.78 %.

(B) $\alpha_{4.4}$-sericin[1]

388 gs. of raw cocoon layers were extracted by boiling for only 10 minutes with 10 *l.* of distilled water, and precipitate at pH 4.4 was brought to the powdered state by means of alcohol and ether.

Yield⋯⋯12.7 gs. N %,⋯⋯17.28 %.

(2) Treatment of $\alpha_{3.8}$-sericin with boiling water and isolation of $\alpha_1$sericin.

20 gs. of powdered $\alpha_{3.8}$-sericin was treated with 5 *l.* of boiling water for 30 minutes. Insoluble part of $\alpha_{3.8}$-sericin war collected on the glass filter and brought to the powdered state by means of alcohol and ether.

Yield⋯⋯10.6 gs. N %.⋯⋯17.13 %.

(3) Treatment of $\alpha_{3.8}$-, $\alpha_{4.4}$-, and α_1-sericin with tannic acid.

0.2 gs. of powdered sericins were treated with tannic acid of 10.00 gs/*l.* concentration, kept at 25°C for 3 hours.

Kind of Sericin	$\alpha_{4.4}$-sericin	$\alpha_{3.8}$-sericin	α_1-sericin
Tannin adsorbed in percentage.	9.92	8.24	10.16

Table clearly shows that adsorption of α_1-sericin with tannic acid is very similar to that of $\alpha_{4.4}$-sericin, and not to $\alpha_{3.8}$-sericin.

(4) Determination of isoelectric point of $\alpha_{4.4}$-, α_1-, and $\alpha_{3.8}$-sericin by dye technic.

Leob[3] showed that acid dye combined with collagen on the acid side of its isoelectric point and basic dye combined with collagen on the alkaline side of its isoelectric point. We used this principle to measure the isoelectric point of sericin. The procedure was as follows ;—

0.3 gs. of dried sericin was kept for 10 hours in 50 cc. of acetate mixture of given pH value (0.02 m) and then for 8 hours in another 50 cc. of buffer containing dye (final dye conc. was equal to 0.005 %). Uncombined dye was deter-

Kind of Sericin	Dyestuff \ pH	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8
$\alpha_{4.4}$-Sericin	Orange G, adsorbed in (%)	—	—	—	0.91	0.84	0.76	0.64	0.57
	Ditto to methylene blue	—	—	—	0.91	0.84	0.76	0.64	0.57
α_1-Sericin	Orange G, adsorbed in (%)	1.20	1.16	1.04	0.96	0.81	0.74	0.58	—
	Ditto to methylene blue	0.37	0.44	0.53	0.71	0.82	0.87	0.88	—
$\alpha_{3.8}$-Sericin	Orange G, adsorbed in (%)	0.92	0.86	0.82	0.77	—	0.71	0.59	—
	Ditto to methylene blue	0.69	0.78	0.82	0.88	—	0.98	1.04	—

mined by colorimetry. Dyestuffs used were Orange G (as acid dye) and methylene blue (as basic dye). Experimental results are shown in the following Table and figs. (see figs. 1, 2 and 3).

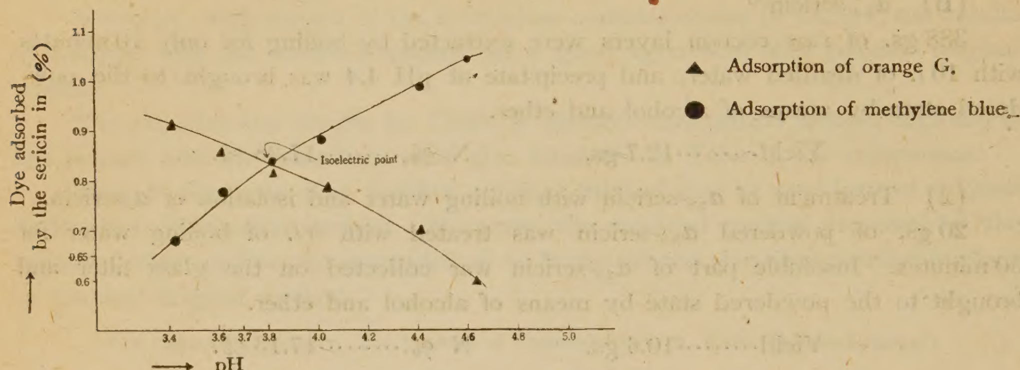


Fig. 1. Determination of Isoelectric Point of $\alpha_{3.9}$-Sericin by the Dye Technic.

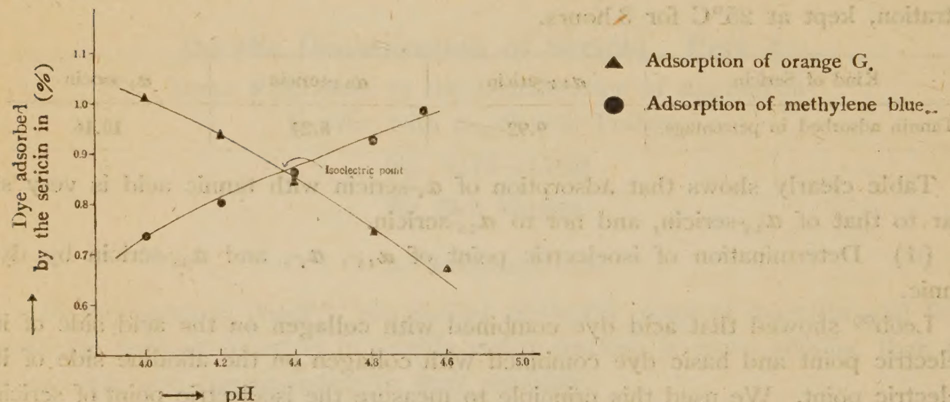


Fig. II. Determination of Isoelectric Point of $\alpha_{4.4}$-Sericin by the Dye technic.

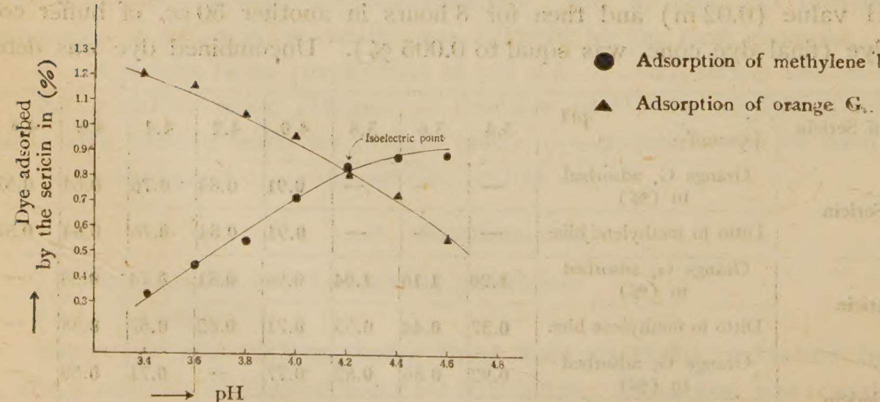


Fig. III. Determination of Isoelctric Point α_1-sericin by the Dye Technic.

(5) Combination of $\alpha_{4.4}$-, α_1- and $\alpha_{3.8}$-sericin with iodine.

0.2 gs. of dried sericins were treated with 50 cc. of 0.079 *N.* iodine, kept at 25 for 3 hours.

Kind of Sericin	$\alpha_{4.4}$-Sericin	$\alpha_{3.8}$-Sericin	α_1-Sericin
Iodine combined per gs. of sericin in gs.	0.088	0.077	0.089

3. Summary.

The work included in this paper may properly be summed up as follows,

(1) $\alpha_{4.4}$-sericin and α_1-sericin takes up more acid dyes and tannic acid than $\alpha_{3.8}$-sericin, while, on the contrary, $\alpha_{3.8}$-sericin takes up more basic dyes than $\alpha_{4.4}$-, and α_1-sericin.

(2) $\alpha_{4.4}$-sericin and α_1-sericin combines more iodine than $\alpha_{3.8}$-sericin, indicating $\alpha_{4.4}$-, and α_1-sericin has more aromatic amino acid(4) and tryptophane than $\alpha_{3.8}$-sericin.

A. With regard to the isoelectric point of $\alpha_{3.8}$-sericin, its isoelectric point is 3.7～3.8, being agreed with the announcement already made by Dr. Ito(2).

B. With regard to the isoelectric point of $\alpha_{4.4}$-sericin its isoelectric point is 4.3～4.4, being agreed with my report already made in the previous paper(1).

C. The isoelectric point of $\alpha_1$sericin is near 4.2. This fact clearly shows, when α-sericin is treated with hot water, insoluble part of α-sericin, or α_1-sericin, is more on the alkaline side than original one.

4. Biblioglaphy.

(1) Hirose: Agr. Chem. Soc. Japan. Part 2. (1940).

(2) Ito and Komori: The Agr. Chem. Soc. Japan. 13 159, 115～116 (1937).

(3) Wilson: Chemistry of Leather Manufacture, Vol. 2, 2nd Edition, New York (1927).

(4) Sutermeister-Brühl: Das Kasein. pp. 15～18 (1932) Berlin.

Studies on the Vitamins of Fish Livers. (Part II.)

Relation Between Age of Fish and Vitamin A Content of Liver Oil.

(pp. 1181～1188)

By Hideo Higashi

(Imperial Fisheries Experimental Station, Tokyo, Japan;
Received November 15, 1940.)

I have observed that in several species of fish, if all conditions except age (or size) are nearly equal, liver oils of older fish are richer in vitamin A than those of younger fish. I believe that the older fish consumes less vitamin A for unit body weight than younger fish. This is the reason why the liver oils of

older fish are richer in vitamin A than those of younger fish. When all conditions, other than age, are nearly equal, the amount of vitamin A consumed per unit of body weight would be proportional to the velocity of growth. So the relation between age of fish and vitamin A content of liver oil can be expressed by a curve related to the growth curve of fish.

According to this assumption, it is easily presumed that the liver oils of very old fish in each species are extraordinarily rich in vitamin A.

Results which I have obtained are as follows :—

No.	Species	Fishing Season	Fishing Ground	Sex	Body Length cm.	Body Wt. g.	Liver Wt. / Body Wt. (%)	Oil Content of Liver (%)	C. L. O. U.
1	Cynopsetta dubia S.	June 28th. 1933	Bering Sea	Female	70.5	4900	3.57	13.2	143
2	Gadus macrocephalus T.	June 25th. 1933	Bering Sea	Female	92.0	12600	3.05	6.98	500
3	Sebastodes flammeus J. and S.	Apr. 10th. 1936	Off the Coast of Shiogama	Male	45.0	2250	1.24	8.30	2240
4	Sebastodes iracundus J. and S.	May 6th. 1936	Off the Coast of Mito	Female	63.0	6200	1.66	15.3	2880
5	Etelis carbunculus C. and V.	Dec. 20th. 1937	Off the Coast of Kagoshima	Male	65.0	6500	0.37	5.70	600
6	Papacaecio caeruleus (K.).	Dec. 20th. 1937	Off the Coast of Kagoshima	Male	39.5	1900	0.36	46.7	350
7	Ocycrius japonicus D.	Dec. 20th. 1937	Off the Coast of Kagashima	Female	64.0	7600	0.78	2.44	900
8	Xiphias gladius L.	Apr. 14th. 1938	Adjacent Sea of Hachijo	Male	178.0	28970	1.17	5.88	450
9	Pristipomoides sieboldi (B.).	Dec. 20th. 1939	Off the Coast of Kagoshima	Female	60.0	4530	0.64	5.26	1260
10	Neothunus macropterus (T. and S.).	Feb. 1st. 1940	Adjacent Sea of Parao	Male	125.0	46500	0.58	2.32	840

Dietary Studies on the Increase of Utilizing Value of Northern Farm Animals. I.

Hair Growth and Feed.

(pp. 1189~1199)

By E. TAKAHASHI and K. SHIRAHAMA.

(Department of Agriculture, Hokkaido Imperial University;
Received November 25, 1940.)

Various kinds of feed were analysed for their cystine contents and a few basic experiments on the relation of the hair growth and feed were carried out on albino rats.

Studies on the Lipids of Salmon Eggs.

(1) On the Acetone Soluble Fraction.

(pp. 1200~1206)

By Kimiko ANNO.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received November 25, 1940.)

Salmon eggs, *Oncorhynchus Gorbuscha,* were extracted with methyl alcohol, petroleum ether and ether. The lipids obtained were separated into phosphatides and fatty oil with acetone.

The fatty oil on saponification gave fatty acids and unsaponifiable matter.

The fatty acids were separated into about 15 per cent of solid and 85 per cent of liquid acids. The solid acid mainly consisted of palmitic acid. The liquid acid contained about half oleic acid and a considerable amount of clupanodonic acid. These acids were isolated and identified. Arachidonic acid probably was present also.

The unsaponifiable matter consisted chiefly of cholesterol.

Sterilizing Action of Acids and Phenols.

(pp. 1207～1224)

By Sogo TETSUMOTO.

(Government Institute for Infectious Diseases, Tokyo Imperial University;
Received November 4, 1940.)

13th Report. Relation between the Chemical Constitution of Phenols and Aromatic Acids and Physiology of Bacteria.

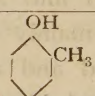
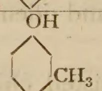
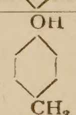
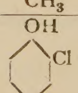
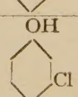
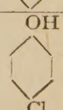
Concerning the relation between the chemical constitution of fatty acids such as normal, iso, cis, trans, d, l, i, meso, and the physiology of bacteria, I have previously reported.

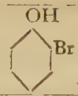
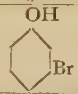
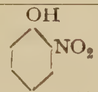
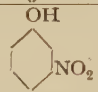
Also concerning the relation between the chemical constitution of phenols such as pyrocatechin (*o*), resorcin (*m*), hydroquinon (*p*), and pyrogallic acid (*o*), phloroglucin (*m*), and the sterilizing action or promoting action, it is reported in my previous paper. Among aromatic acids there are many isomers having different chemical constitutions.

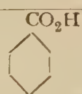
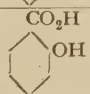
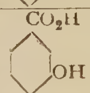
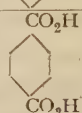
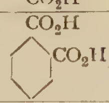
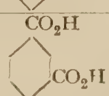
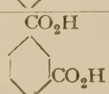
To find what relation exists between the various isomers having different chemical constitutions and the physiology of bacteria, I performed the next experiment. Reagents used are shown in the following table.

§ 1. Reagents.

Table I. Reagents and constitution formulae.

Phenols	Isomer	Chemical constitution	M. P.	B. P.
Cresol $C_6H_4 \cdot CH_3 \cdot OH$ M. W. 108.064	*o*		30°	191°
	m		44°	203°
	p		36°	138°
Chlorophenol $C_6H_4 \cdot Cl \cdot OH$ M. W. 128.530	*o*		7°	175～176°
	m		28.5°	212°
	p		37°	217°

Bromophenol $C_6H_4 \cdot Br \cdot OH$ M. W. 173.030	*o*			195∼198°
	m		32∼33°	236°
	p		64°	238°
Nitrophenol $C_6H_4 \cdot NO_2 \cdot OH$ M. W. 139.078	*o*		45°	214°
	m		96°	194°
	p		114°	

Aromatic acids	Isomer	Chemical constitution	M. P.
Benzoic acid $C_6H_5 \cdot CO_2H$ M. W. 122.048			121°
Salicylic acid $C_6H_4 \cdot OH \cdot CO_2H$ M. W. 138.048	*o*		156°∼157°
Mata-oxybenzoic acid	*m*		188°
Paraoxybenzoic acid	*p*		213°
Phthalic acid $C_6H_4\begin{matrix}\diagup CO_2H \\ \diagdown CO_2H\end{matrix}$ M. W. 166.048	nor		196°∼199°
	iso		332°∼335°
	tele		over 300° sublimes

§ 2. Relation between the chemical constitutions of phenols and aromatic acids and the sterilizing action at the same concentration.

To find what relation exists between the chemical constitutions of phenols and aromatic acids and the sterilizing action on bacteria, I performed this experi-

ment. Concentration of each reagent was made $N/1000$, only phthalic acids were made $N/100000$, because they are hardly soluble in water. Results obtained are as shown in the following tables.

Table 2. Relation between the chemical constitution of phenols and the strength of sterilizing action.

Phenols	Isomer	pH	Surviving period			
			Staph. pyog.	P. vulgar.	B. typhosus	V. cholerae.
Cresol	*o*	5.36	$6^d + 7^d -$	$4^d \pm 5^d -$	$5^d + 6^d -$	$6^h \pm 9^h -$
	m	〃	$7 + 8 -$	$5 + 6 -$	$7 \pm 8 -$	$9 + 12 -$
	p	5.57	$5 + 6 -$	$5 + 6 -$	$3 + 4 -$	$3 + 6 -$
Cl-phenol	*o*	5.73	$4 + 5 -$	$2 + 3 -$	$3 + 4 -$	$60^m + 90^m -$
	m	5.76	$3 \pm 4 -$	$24^h + 36^h -$	$2 + 3 -$	$45 + 60 -$
	p	〃	$2 + 3 -$	$18 + 24 -$	$36^h \pm 2 \pm$	$30 + 45 -$
Br-phenol	*o*	5.76	$3 \pm 4 -$	$24 + 36 -$	$2^d + 3^d -$	$45 \pm 60 -$
	m	5.80	$2 + 3 -$	$18 + 24 -$	$24^h + 36^h -$	$30 + 45 -$
	p	〃	$36^h + 2 -$	$12 + 18 -$	$18 + 24 -$	$20 + 30 -$
NO_2-phenol	*o*	5.76	$2^d + 3^d -$	$24 \pm 36 -$	$2^d \pm 3^d -$	$30 \pm 45 -$
	m		$24^h + 36^h -$	$12 + 18 -$	$18^h + 24^h -$	$20 + 30 -$
	p	5.78	$18 + 24 -$	$9 + 12 -$	$12 + 18 -$	$15 + 20 -$
Control			$8^d \pm$	$5^d \pm$	$6^d \pm$	$18^h \pm$

Table 3. Relation between the chemical constitution of aromatic acids and the strength of the sterilizing action. ($N/1000$, only phthalic acids $N/100000$)

Acid	Isomer	pH	Surviving period			
			Staph. pyog.	P. vulgar.	B. typhos.	V. choler.
Benzoic		3.58	$12^h + 24^h -$	$6^h \pm 9^h -$	$9^h + 12^h -$	$20^m + 30^m -$
Salicylic	*o*	3.08	$90^m + 2^h -$	$45^m + 60^m -$	$60^m + 90^m -$	$2.5^m \pm 5^m -$
m-OH-benz.	*m*	3.68	$9^h + 12 -$	$6^h \pm 9^h -$	$6^h + 9^h -$	$20^m \pm 30^m -$
p-OH-benz.	*p*	〃	$9 \pm 12 -$	$3 + 6 -$	$6 \pm 9 -$	$15 + 20 -$
Phthalic	nor	4.34	$2^d + 3^d -$	$36^h + 2^d -$	$2^d \pm 3^d -$	$90^m \pm 2^d -$
	iso	4.83	$3 + 4 -$	$2^d \pm 3^d -$	$2 + 3 -$	$2^h + 3^h -$
	tele	4.13	$4 + 5 -$	$3 \pm 4 -$	$3 + 4 -$	$3 + 6 -$
Control			$8^d \pm$	$5^d \pm$	$6^d \pm$	$18^h \pm$

From the results obtained I found the following facts. The sterilizing action of p isomers is the strongest of all the phenols. In cresols the degree of the strength of the sterilizing action is as follows :— $m<o<p$.

Among halogen phenols and NO_2 phenols the strength of the sterilizing action is as follows :— $o<m<p$.

The sterilizing action of phenols has no relation to pH of each phenol. The sterilizing action of O-OH-benzoic acid is the strongest among OH benzoic acid isomers. The order of the strength of OH benzoic acid isomers is as follows :— $m<p<o$.

The chief cause of difference of the sterilizing action is based on pH and partly on each position of OH group combined at benzene ring. Among phthalic acid isomers the order of the strength of the sterlizing action is as follows :—

tele < iso < normal.

And pH of each isomer is as follows :– tele < normal < iso.

Accordingly there seems to exist no relation between the sterilizing action of each isomer and pH.

§ 3. The action of salts and anions of phenol isomers and aromatic acid isomers on the physiology of bacteria.

To examine how the anions of phenol isomers and aromatic acid isomers act on microorganisms, I made aqueous solution of Na, Ca, and NH_4 salts, each having the same anions as each phenol isomer or aromatic acid isomer respectively, and performed this experiment. Except phthalic acid salts the concentration of salts was made $N/1000$. Concentration of phthalic acid salts was made $N/100000$.

Table 4. The action of neutral salts of phenols and aromatic acids.

I. Na salts of phenols.

Na–	Isomer	Surviving period			
		Staph. pyogen.	Prot. vulgar.	Bac. typhos.	Vib. choler.
Cresolate	o	$7^d - 9^d$	$5^d - 6^d$	$6^d - 7^d$	$9^h + 12^h -$
	m	$9 - 11$	$6 - 7$	$8 - 10$	$24 + 36 -$
	p	$6 - 7$	$4 - 5$	$5 - 6$	$6 + 9 -$
Cl-phenolate	o	$6^d \pm 7^d -$	$4 + 5 -$	$5^d \pm 6^d -$	$6 + 9 -$
	m	$4 + 5 -$	$3 + 4 -$	$3 + 4 -$	$5 + 8 -$
	p	$3 + 4 -$	$2 + 3 -$	$2 + 3 -$	$3 + 5 -$
Br-phenolate	o	$5 \pm 6 -$	$2 + 3 -$	$4 + 5 -$	$3 + 6 -$
	m	$3 \pm 4 -$	$36^h + 2 -$	$2 + 3 -$	$2 + 3 -$
	p	$2 + 3 -$	$24^h + 36^h -$	$2 \pm 3 -$	$90^m + 2 -$

NO_2-phenolate	*o*	4 + 5 −	$2^d \pm 3^d$ −	3 + 4 −	2^h + 3 −
	m	3 ± 4 −	36^h + 2 −	2 + 3 −	90^m + 2 −
	p	2 + 3 −	$24^h \pm 36^h$ −	$24^h + 36^h$ −	$60^m + 90^m$ −
Control		8^d ±	5^d ±	6^d ±	18^h ±

II. Na salts of aromatic acids.

Na−	Isomer	Surviving period			
		Staph. pyogen.	Prot. vulgar.	Bac. typhos.	Vib. choler.
Benzoate		$15^d - 18^d$ −	$8^d - 10^d$	$10^d - 13^d$	$18^h + 24^h$ −
Salicylate	*o*	4 − 5	2 − 3	3 − 4	$30^m + 45^m$ −
m-o-benzoate	*m*	10 −13	6 − 8	8 −10	$12^h + 24^h$ −
p-o-benzoate	*p*	8 −10	5 − 7	6 − 8	9 +12 −
Phthalate	nor	15 −20	8 −10	12 −15	9 ±12 −
	iso	20 −25	10 −13	15 −18	12 +18 −
	tele	25 −30	17 −20	20 −25	18 +24 −
Control		8^d ±	5^d ±	6^d ±	18^h ±

Since the results of Na, Ca, and NH_4 salts were nearly the same, I have described the results of Na salts only.

From the results above noted, we can deduce these facts:

(1) The order of preventing power for the survival of bacteria is as follows.

a. Salts of cresol isomers, $m < o < p$.

b. Salts of halogen phenol and NO_2 phenol isomers, $o < m < p$.

c. Salts of OH substituted benzoic acid isomers, $m < p < o$.

d. Salts of phthalic acid isomers, tele<iso<normal.

From these facts we can deduce the following:

(2) Among cresol isomers, only anion of para isomer has the preventing power for bacteria, but anions of *o* and *m* have no such power.

(3) The strength of preventing power of anions of halogen phenol and NO_2 phenol for the survival of bacteria is as follows: $o < m < p$.

(4) Among anions of benzoic acid and its OH substituted acids, only anions of O-OH-benzoic acid (salicylic acid) have the preventing action on the survival of bacteria, but other anions such as *m* or *p* have none.

(5) Anions of phthalic acid isomers have no preventing action.

§ 4. Sterilizing action of phenols and aromatic acids isomers at the same pH solution.

To find the relation between the strength of sterilizing power of *o*, *m*, and *p* isomers of phenols or aromatic acids and the chemical constitution of each reagent,

I made an aqueous solution of each reagent, making the aqueous solution of pH 5.45 with cresols and that of pH 5.80 with halogen phenols and NO_2 phenols at

Table 5. Effects of *o*, *m*, and *p* phenol and aromatic acid isomers on the physiology of bacteria at the same pH.

I. Phenols.

Phenols	Isomer	Concent.	pH	Surviving period			
				Staph. pyogen.	Prot. vulgar.	Bac. typhos.	Vib. choler.
Cresol	*o*	*N*/2000	5.45	$6^d + 7^d -$	$5^d + 6^d -$	$6^d + 7^d -$	$6^h + 9^h -$
	m	〃	〃	$8 + 9 -$	$6 + 7 -$	$7 + 8 -$	$12 \pm 18 -$
	p	*N*/1000	〃	$5 + 6 -$	$3 \pm 4 -$	$3 + 4 -$	$3 + 6 -$
Cl-phenol	*o*	*N*/2000	5.80	$5 + 6 -$	$3 + 4 -$	$4 + 5 -$	$90^m + 2^h -$
	m	*N*/1500	〃	$3 + 4 -$	$36^h \pm 2 -$	$2 + 3 -$	$60 + 90^m -$
	p	〃	〃	$2 + 3 -$	$24 + 36^h -$	$36^h + 2^d -$	$30 + 45 -$
Br-phenol	*o*	*N*/1500	〃	$3 + 4 -$	$36 \pm 2^d -$	$2^d + 3 -$	$45 + 60 -$
	m	*N*/1000	〃	$2 + 3 -$	$18 + 24^h -$	$24^h + 36^h -$	$30 + 45 -$
	p	〃	〃	$36^h + 2 -$	$12 + 18 -$	$18 + 24 -$	$20 + 30 -$
NO_2-phenol	*o*	*N*/1500	〃	$3^d \pm 4 -$	$24 + 36 -$	$2^d + 3^d -$	$30 + 45 -$
	m	〃	〃	$36^h + 2 -$	$12 + 18 -$	$24^h + 36^h -$	$20 + 30 -$
	p	*N*/1000	〃	$18 + 24^h -$	$9 + 12 -$	$12 + 18 -$	$15 + 20 -$
Control				$8^d \pm$	$5^d \pm$	$6^d \pm$	$18^h \pm$

II. Aromatic acids.

Acid	Isomer	Concent.	pH	Surviving period			
				Staph. pyogen	Prot. vulgar.	Bac. typhos.	Vib. choler.
Benzoic		*N*/1100	3.68	$18^h \pm 24^h -$	$6^h + 9^h -$	$12^h + 18^h -$	$20^m + 30^m -$
Salicylic	*o*	*N*/6000	〃	$3 + 6 -$	$90^m + 2^h -$	$2 + 3 -$	$10 + 15 -$
m-o-benzoic	*m*	*N*/1000	〃	$9 + 12 -$	$6 \pm 9 -$	$6 + 9 -$	$20 \pm 30 -$
p-o-benzoic	*p*	〃	〃	$9 \pm 12 -$	$3 + 6 -$	$6 \pm 9 -$	$15 + 20 -$
Phthalic	nor	*N*/11000	4.38	$2^d + 3^d -$	$36^h + 2^d -$	$2^d \pm 3^d -$	$90^m + 2^h -$
	iso	*N*/10000	〃	$3 + 4 -$	$2^d + 3^d -$	$3 \pm 4 -$	$2^h + 3 -$
	tele	*N*/300000	〃	$6 + 7 -$	$4 + 5 -$	$5 + 6 -$	$9 + 12 -$
Control				$8^d \pm$	$5^d \pm$	$6^d \pm$	$18^h \pm$

2.00 respectively. Then I examined the relation betweem the chemical constitution of cresols, halogen phenols, NO_2 phenols and aromatic acids and the strength of sterilizing action at the same pH solution respectively. Results obtained are as shown in Table 5.

From the above experiments noted in Table 5, I learned the following facts:

1. In the same pH solution the strength of the sterilizing power of cresol is as follows: $m<o<p$.

The strength of the sterilizing action of cresol anion is as follows:

Anions of *p* cresol have the preventing power for the bacteria but *o* or *m* anion has no such power. The variation in the strength of the sterilizing or preventing action of *o*, *m*, or *p* cresol isomer on the bacterial life depends chiefly on the position of CH_3 group combined at benzene ring, but has no relation to pH.

2. The strenghth of the sterilizing power of halogen phenols and NO_2 phenols in the same pH solution is as follows: $o<m<p$.

The difference between the sterilizing action and the constitution of halogen phenol or NO_2 phenol, chiefly depends on the position of Cl_2, Br_2, or NO_2 group combined at benzene ring.

3. When we compare the sterilizing action of benzoic acid and *o*, *m*, and *p* isomers of OH substituted benzoic acid in the same pH solution, we find that there seems to be a great difference in the case of halogen phenols and NO_2 phenols. The order of the sterilizing action is as follows:—benzoic acid$<$*m*-OH benzoic acid$<$*p*-OH benzoic acid$<$*o*-OH benzoic acid. The chief cause of this fact is that the strong sterilizing action of salicylic acid is chiefly due to the low pH, and partly that anion of salicylic acid has sterilizing action. On the other side *o*-OH benzoic acid and *m*-OH benzoic acid have high pH compared to salicylic acid and their anions have no sterilizing action on bacteria.

4. If we compare the sterilizing action of 3 isomers of phthalic acids, such as nor., iso and tele, we see that the degree of the sterilizing power is nor.$>$iso$>$tele. This is due to the chemical constitution of undissociated molecule of each isomer, chiefly position of CO_2H group combined at benzene ring.

§ 5. Summary and discussion concerning the relation between the chemical constitution of phenols and aromatic acids and the physiology of bacteria.

From the results mentioned in the sections (2) to (4), we obtained the following views on the relation between the chemical constitution of phenols and aromatic acids and the sterilizing action on bacteria.

1. The sterilizing action of *o*, *m*, and *p* isomers of phenols, in the same concentration or in the same pH solution, is as follows:

The strongest of all is *p* isomer, and *o* isomer is the weakest. e.g., $p>m>o$. But with cresol, the order of the strength of the sterilizing action is as follows: $p>o>m$.

And among OH substituted benzoic acids, the order of the strength of the sterilizing action is as follows:—

o-OH benzoic acid>p-OH benzoic acid>m-OH benzoic acid>benzoic acid. And in the solution of phthalic acid isomer, normal>iso>tele.

2. The cause of the difference of the sterilizing action of *o*, *m*, and *p* cresol isomer on the bacterial life seems chiefly to depend on the poisoning action for bacterial body by the position of CH_3 group combined at benzene ring. While the difference of the sterilizing action of *o*, *m*, and *p* OH benzoic acids depends chiefly on pH which is changed by the position of OH group combined at benzene ring. Added to this, the difference of the action of undissociated molecules of acid isomers also have some effects on the sterilizing action.

3. The strength of the sterilizing action of phthalic acids in the solution of the same concentration and also in the same pH is as follows:

tele<iso<normal.

The cause of the difference of the strength of each acid isomer depends on the position of CO_2H combined at benzene ring.

4. Judging from the results of the sterilizing action of phenols, OH benzoic acids and phthalic acids, we ascertained the following facts: Anions of phenols, OH benzoic acids and phthalic acids, have generally no sterilizing action or almost no preventing action on bacteria. Only anions of halogen phenols and NO_2 phenols and o-OH benzoic acid have a weak preventing action.

14th Report. On the Relation between the Chemical Constitution of Phenol Isomers and Aromatic Acid Isomers and Adsorption in the Bacterial Body.

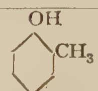
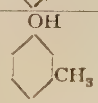
Concerning the adequate relation between the strength of the sterilizing action of several phenols and aromatic acids and the adsorption on the bacterial body, we see many reports. But there seems to be no study concerning the difference of chemical constitution having a special effect on the life of bacteria.

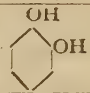
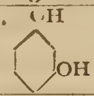
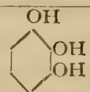
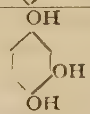
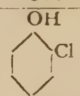
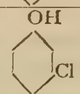
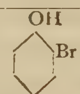
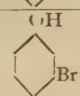
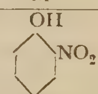
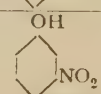
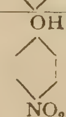
I performed this experiment to find how the difference of chemical constitution of phenols, OH benzoic acid isomers and phthalic acid isomers act on the protoplasm of the bacterial body.

(1) Reagents.

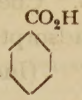
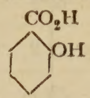
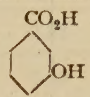
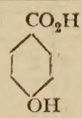
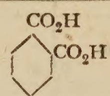
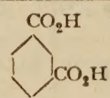
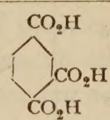
Reagents used are as follows:

I. Phenols.

Phenols	Rational formulae, / Molecular weight	Isomer	Constitution formulae	M. P.	B. P.
		o-		30°	191°
Cresol	$C_6H_4 \cdot CH_3 \cdot OH$ 108.064	m		44°	203°

		p		36°	138°
Pyrocatechin	$C_6H_4(OH)_2$ 110.048	*o*		104°	
Resorcin		*m*		110°	
Hydroquinon		*p*		170°	
Pyrogallic	$C_6H_3(OH)_3$ 126.048	*o*		132°	
Phloroglucin		*m*		218°	
Cl-phenol	$C_6H_4 \cdot Cl \cdot OH$ 128.530	*o*		7°	175°~176°
		m		28.5°	212°
		p		37°	217°
Br-phenol	$C_6H_4 \cdot Br \cdot OH$ 173.030	*o*			195°~198°
		m		32°~38°	236°
		p		64°	238°
NO_2-phenol	$C_6H_4 \cdot NO_2 \cdot OH$ 139.078	*o*		45°	214°
		m		96°	194°
		p		114°	

II. Aromatic acids.

Aromatic acid	Rational formulae / Molecular weight	Isomer	Constitution formulae	M. P.
Benzoic	$C_6H_5 \cdot CO_2H$ 122.048			121°
Salicylic		*o*		156°~157°
m-OH-benzoic	$C_6H_5 \cdot OH \cdot CO_2H$ 138.048	*m*		188°
p-OH-benzoic		*p*		213°
		nor		196°~199°
Phthalic	$C_6H_4\langle^{CO_2H}_{CO_2H}$ 166.048	iso		332°~335°
		tele		over 300° sublimes

The adsorption state of reagents on bacterial body can be shown by quantitative analysis of each reagent before and after the experiment. But each reagent is acid and then pH of reagent changes proportionally to the quantity consumed by the bacterial body. Accordingly we can find the adsorption state in the bacterial body by measuring the pH of each reagent before and after the experiment.

(2) Experiment.

I used Bac. coli communis, to represent Bac. typhosus, and Vib. cholerae. I gathered each colony from slant cultures after repeating 24 hour culture for 3 days continuously at 37°. I mixed each colony homogeneously in sterilized mortars and I put 1 gram of each colony in 100 c.c. of each reagent and shook it violently in a glass vessel at 37°. After 24 hours, I separated the clear solution from the precipitate by a super electric centrifuge. Taking a certain quantity of each

clear solution determined its pH by electric method and compare the result with the original pH of each reagent.

Results obtained are as shown in the following table.

Table 3. Chemical constitution of phenols and adsorption on the bacterial body. (Increasing value of pH).

Phenols	Isomer	Original pH	Increasing value of pH	
			Coli communis	Vib. cholerae
Cresol	*o*	5.36	1.03	1.12
	m	5.36	0.85	0.98
	p	5.57	1.06	1.10
Pyrocatechin	*o*	5.31	1.13	1.13
Resorcin	*m*	5.57	1.27	1.35
Hydroquinon	*p*	5.64	1.25	1.28
Pyrogallic acid	*o*	4.58	1.16	1.20
Phloroglucin	*m*	5.71	1.25	1.30
Cl-phenol	*o*	5.73	0.45	0.47
	m	5.76	0.60	0.49
	p	5.76	0.80	0.89
Br-phenol	*o*	5.76	0.63	0.79
	m	5.80	0.75	0.85
	p	5.80	0.93	1.15
NO_2-phenol	*o*	5.76	0.65	0.87
	m	5.76	0.85	1.17
	p	5.78	0.95	1.33

Table 4. Chemical constitution pf aromatic acids and adsorption on the bacterial body, as shown. by increasing pH.

Aromatic acids	Isomer	Original pH	Increasing pH	
			Coli communis	Vib. cholerae
Benzoic		3.58	1.58	1.85
Salicylic	*o*	3.08	2.92	3.05
m-OH-benzoic	*m*	3.68	1.85	2.79
p-OH-benzoic	*p*	:	2.32	2.82
Phthalic	normal	4.34	2.32	2.38
	iso	4.38	1.97	2.04
	tele	4.13	1.79	1.89
Cont. 1. HNO_3	*N*/10000	4.0	2.14	2.35
Cont. 2. H_2SO_4	:	:	:	:
Cont. 3. H_2O		6.33	0.70	0.73

Note: Concentration :—*N*/1000. Phthalic acids=*N*/100000. HNO_3 and H_2SO_4=*N*/10000.

(3) Discussion and summary of adsorption of aromatic acid isomers and phenol isomers on bacterial bodies.

Relation between the chemical constitution of phenols and aromatic acids and the bacterial life will be shown exactly by studies on the chemical constitution of reagents and the sterilizing, preventing and promoting actions for bacteria. And these three actions have adequate relation to the adsorption on or consumption by the bacterial protoplasma of reagents. I performed this experiment to ascertain how the difference of the chemical constitution of reagents acts on the adsorption on or consumption by the bacterial protoplasma.

By the results noted in the previous section, we can deduce the following conclusions :

(1) The degree of the strength of sterilizing action of phenol isomers and aromatic acid isomers is proportionate to the degree of the quantity adsorbed on the bacterial body. The degree of adsorption has an adequate relation to the position of OH group, Cl and Br or NO_2 group combined at benzene ring in phenol isomers, and CO_2H group combined at benzene ring in OH benzoic acid isomers, respectively.

(2) On the other hand we see that the action of di and tri OH phenol isomers on the bacteria is as follows : *p* and *o* isomers have a sterilizing action and order of the strength is as follows : $o<p$. Contrary to this, *m* isomers have a strong promoting action for bacteria. *m* isomers seem to be a nutritive source for bacteria.

(3) The cause of the difference of the sterilizing action of phthalic acid isomers is as follows: We see the difference of the quantity adsorbed on the bacterial body among normal, iso and tele isomers, by the position of CO_2H group combined at benzene ring of phthalic acid.

The difference in the amount of adsorption on bacterial bodies causes the difference of the sterilizing action of phthalic acid isomers.